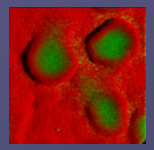
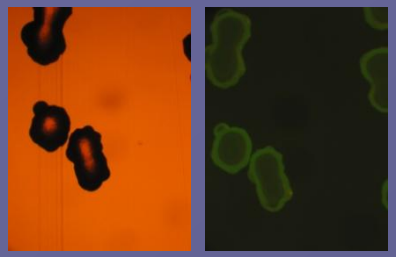
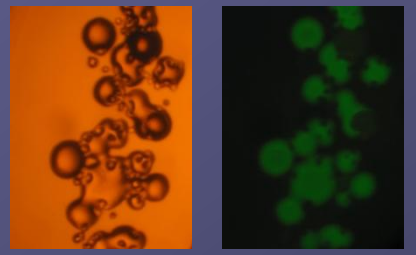


Biofilm formation and antibiotic tolerance: is there a connection

Anders Folkesson

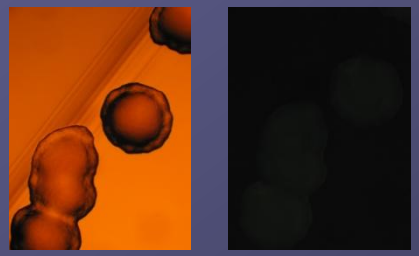
IMG
Infection Microbiology Group
DTU Biosys
The TECHNICAL UNIVERSITY of DENMARK



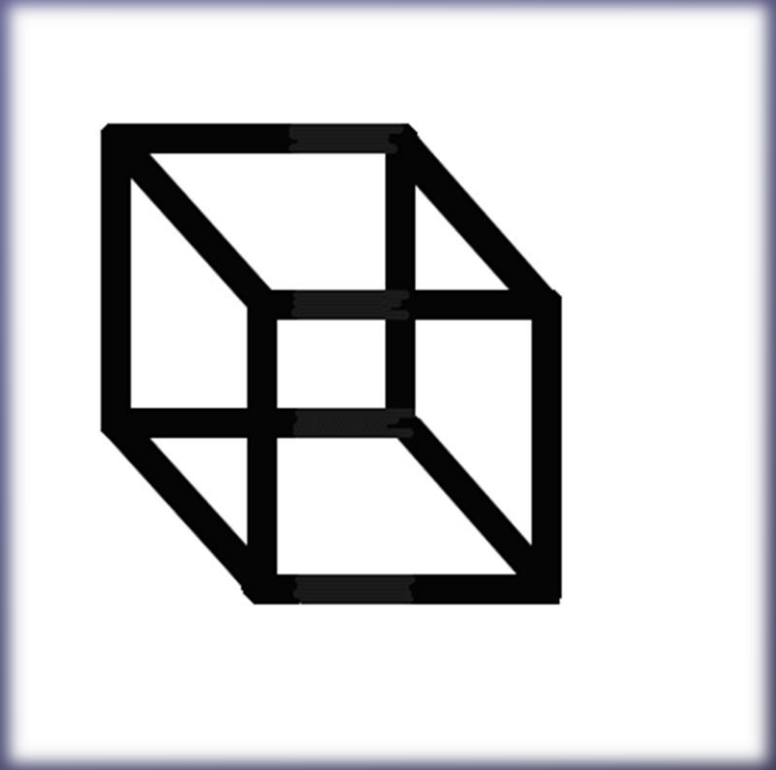


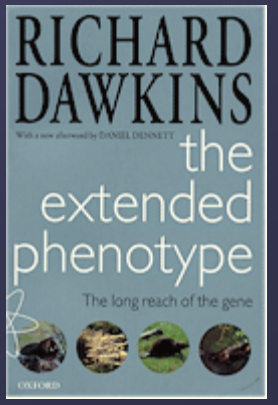
Jacob Olsen
Andreas Arnung
Christian Skjødt

J.A.J Haagensen
A.Reisner
C.Zampaloni
C. Sternberg
S.Molin



The Necker cube.





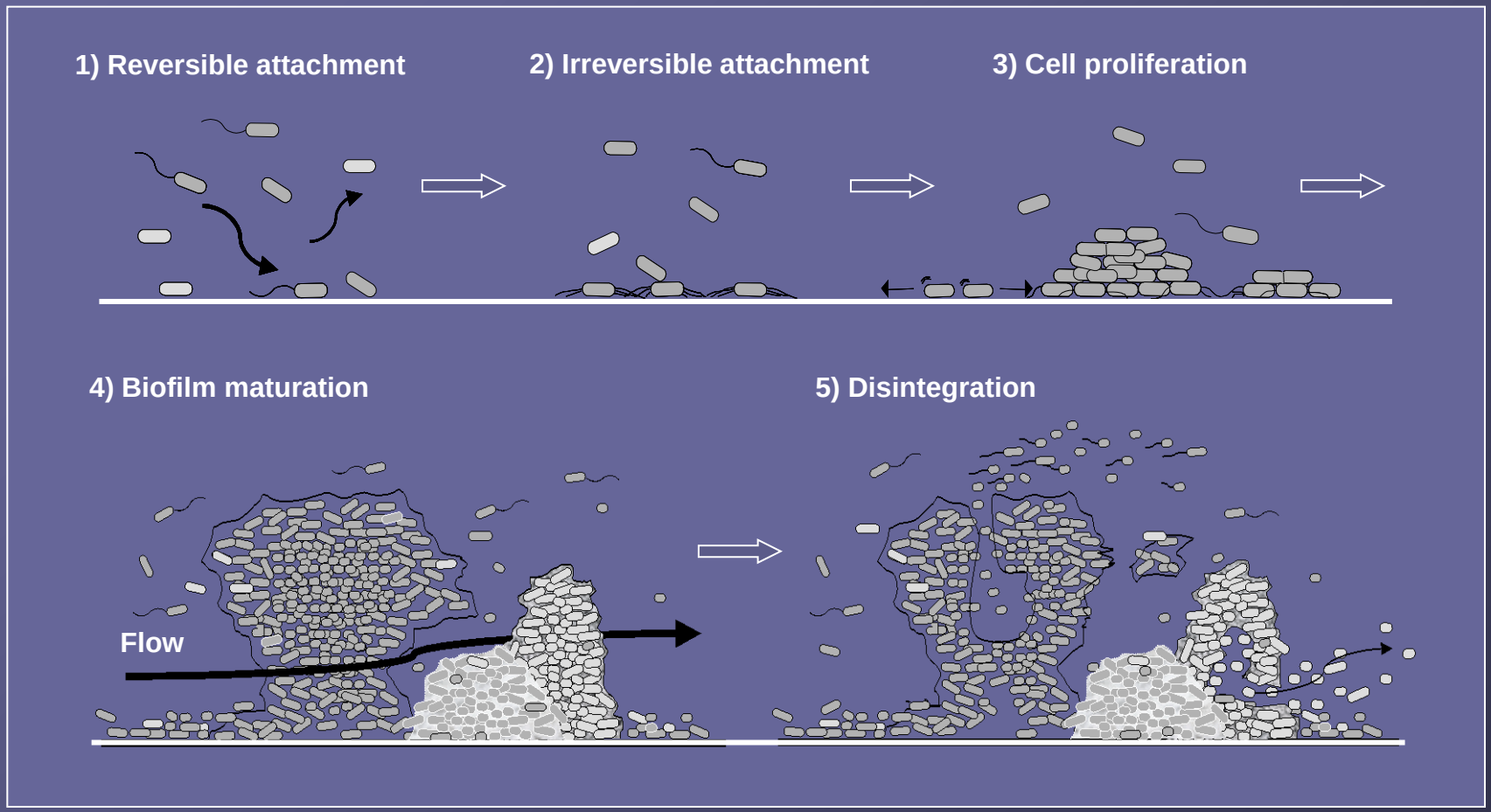
Biofilm formation

Biofilms and antibiotics

***E.coli* as a model system**

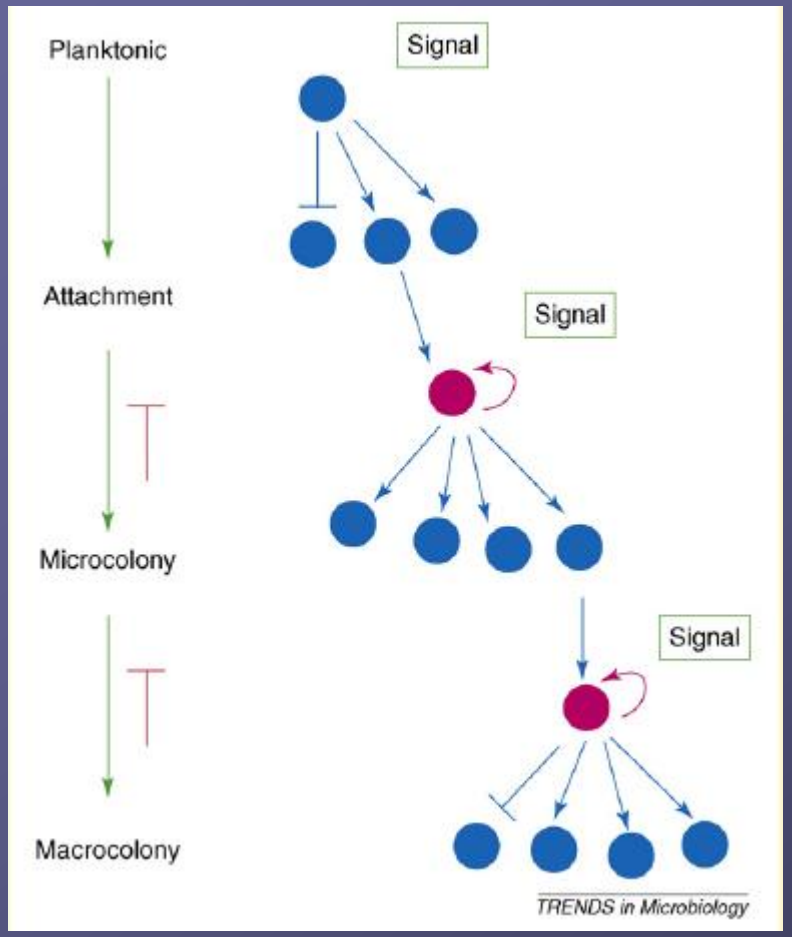
The dynamics of Ab action in microbial biofilms

The classical model for biofilm formation.



A model of the stages of bacterial biofilm development. At stage 1, the bacterial cells attach reversibly to the surface. Then, at stage 2, the cells attach irreversibly, a step mediated mainly by exopolymeric substances, and the cells lose their flagella-driven motility. At the next stage (3), the first maturation phase is reached, as indicated by early development of biofilm architecture. The second maturation phase is reached at stage 4 with fully mature biofilms, as indicated by the complex biofilm architecture. At the dispersion stage (5), single motile cells (dark cells on the figure) disperse from the microcolonies

The classical model for biofilm formation.

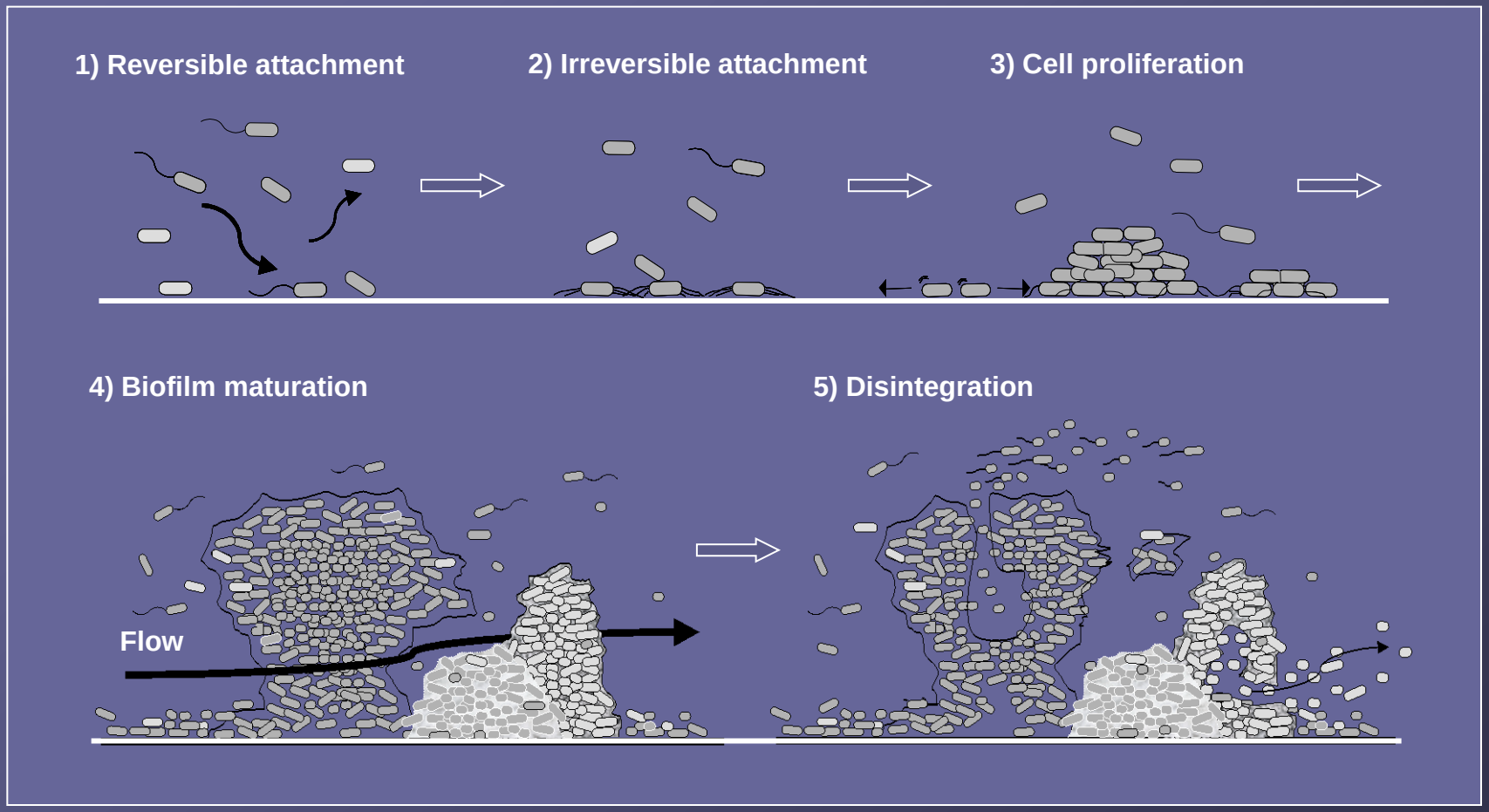


Developmental pathways have evolved as dedicated systems for regulation of biofilm formation.

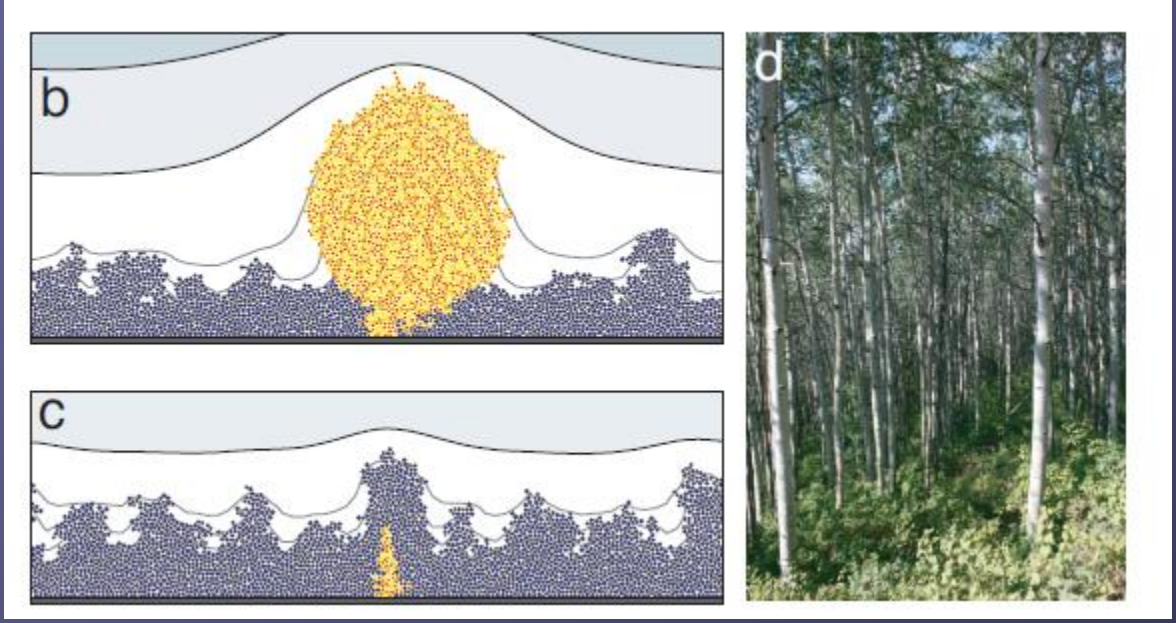
The developmental model describes biofilm formation in terms of a multicellular process, wherein genetic pathways are presumed to have evolved to facilitate cooperation among members of the biofilm

Monds et.al.2009

The not so classical model for biofilm formation.



Individual based modelling of biofilm formation.



Xavier PNAS 2007

How can we differentiate between the two models?

Does it matter, anyway?

Increased tolerance to antimicrobial agents is an important feature of microbes growing in biofilms.

Infections caused by bacterial biofilms
can be persistent and difficult to eradicate.

Susceptibility of planktonic and biofilm bacteria to selected antibiotics

Organism	Antibiotic	Planktonic (μg/ml)	Biofilm (for 99% reduction)
S. aureus	Vancomycin	2 (MBC)	20 (μg/ml)$_{\text{siliconedisc}}$
P. aeruginosa	Imipenem	1 (MIC)	> 1024 (μg/ml)
E. coli	Ampicillin	2 (MIC)	512 (μg/ml)$_{\text{calgary}}$
P. pseudomallei	Ceftazidime	8 (MBC)	800 (μg/ml)$_{\text{robbins}}$
S. sanguis	Doxycycline	0.063 (MIC)	3.15 (μg/ml)$_{\text{robbins}}$

Adapted from Donlan *et al. Clin. Microbiol. Rev.* 2002

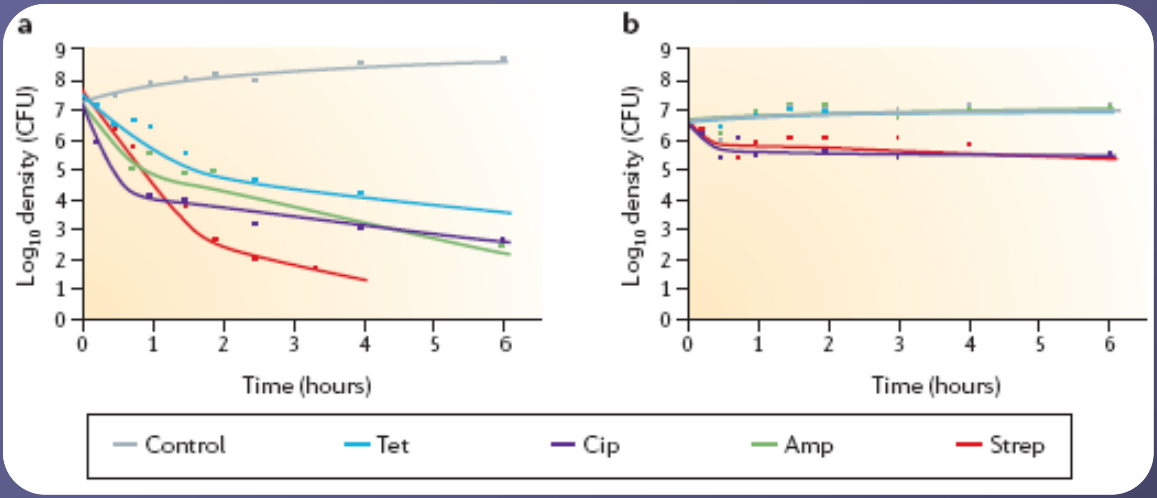
Ab^r Planktonic < Ab^r device.

Bacteria form biofilm in device.

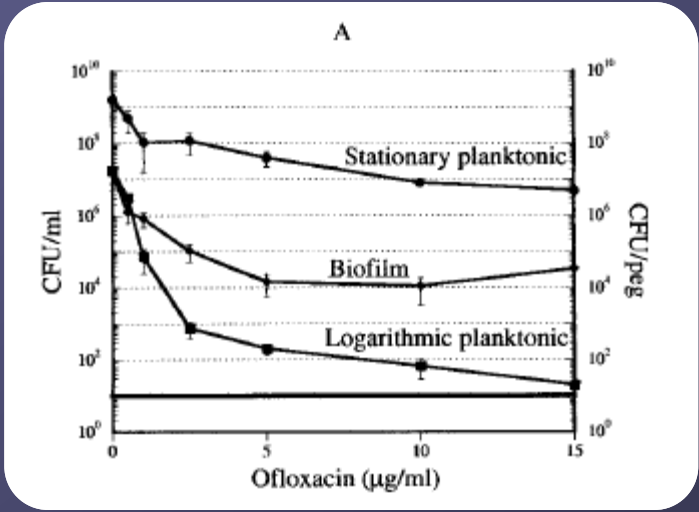
Therefore are biofilms more resistant.

Is a causal relationship established?

Biofilm vs stationary phase as an explanation for the failure of AB treatment without resistance.

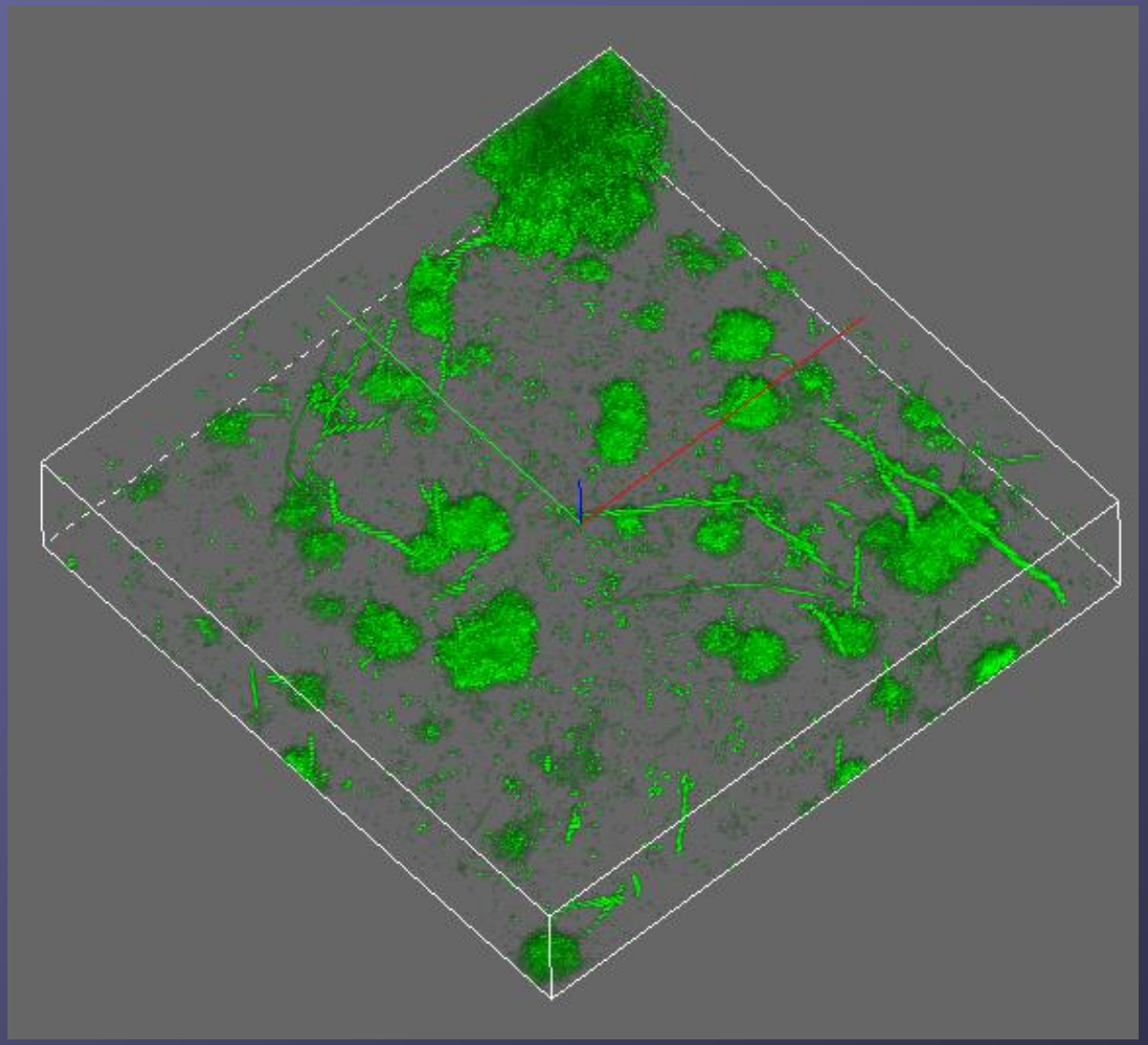


Levin et.al 2006

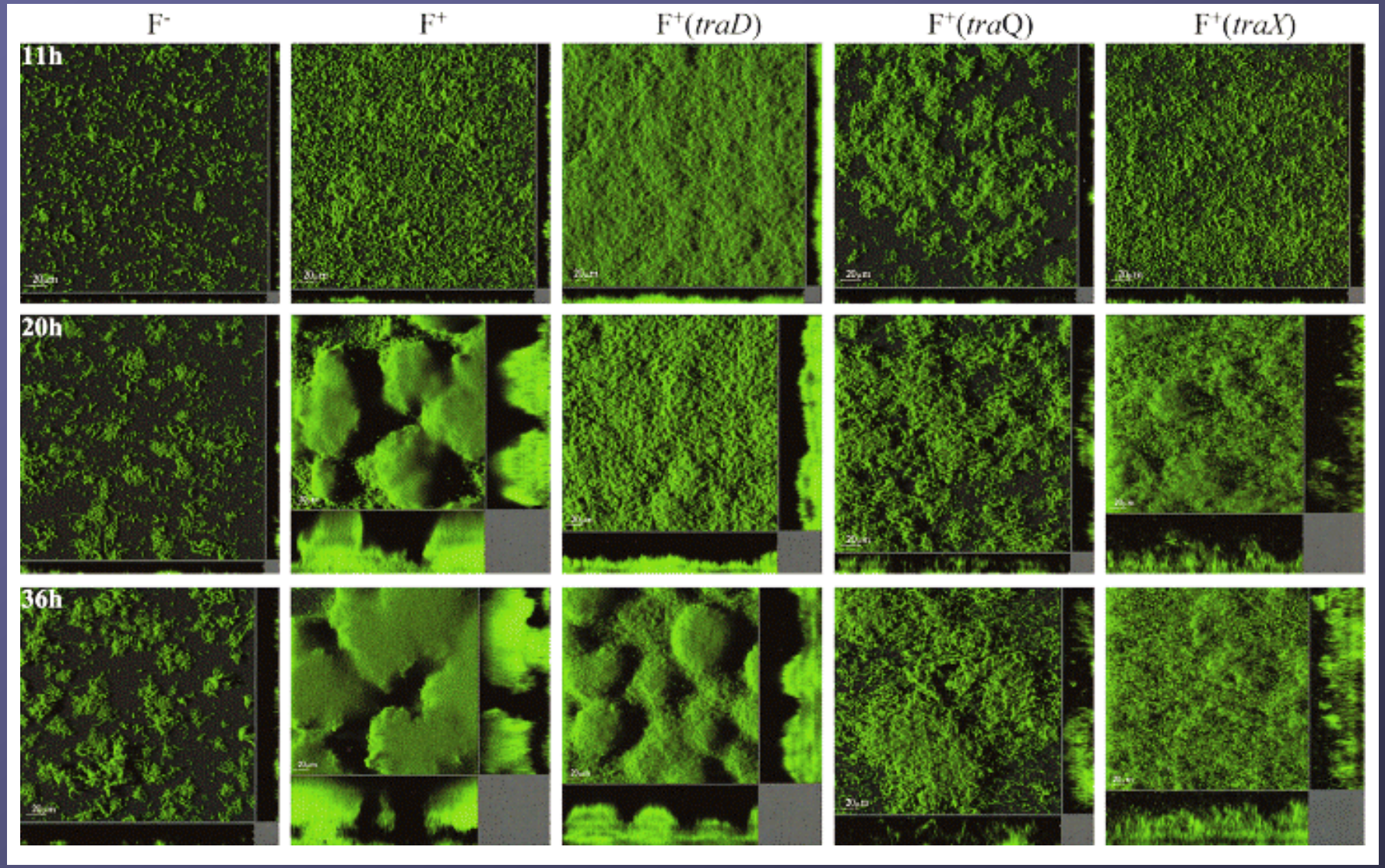


Spoering et.al 2001

Escherichia coli is not an efficient biofilm former.



The spatial distribution of *E. coli* biofilms.



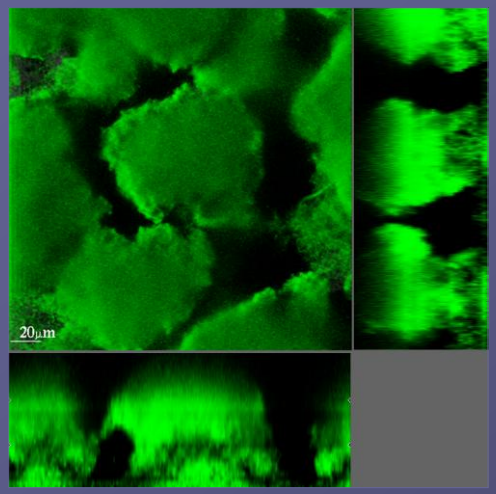
Reisner *et al. Mol Microbiol 03*

The only factor needed for induction of the mushroom state is the pili.

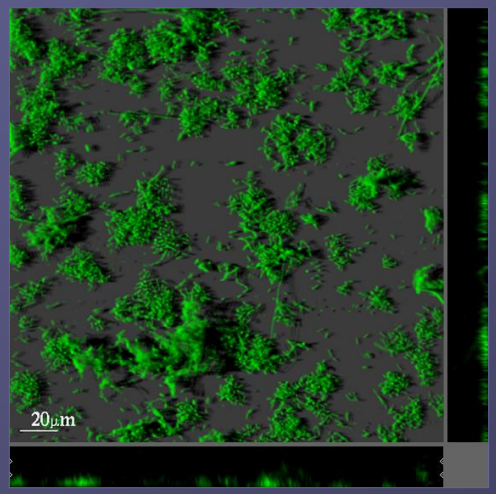
A set of isogenic strains.

Biofilm effect under identical conditions.

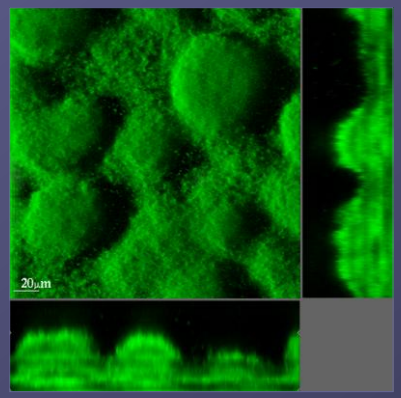
F$^+$



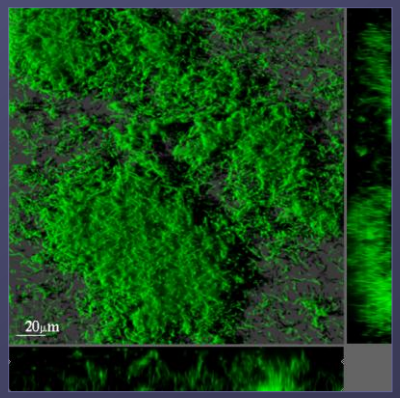
F*traA*



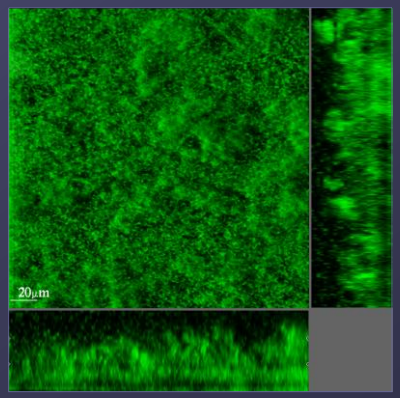
F*traD*



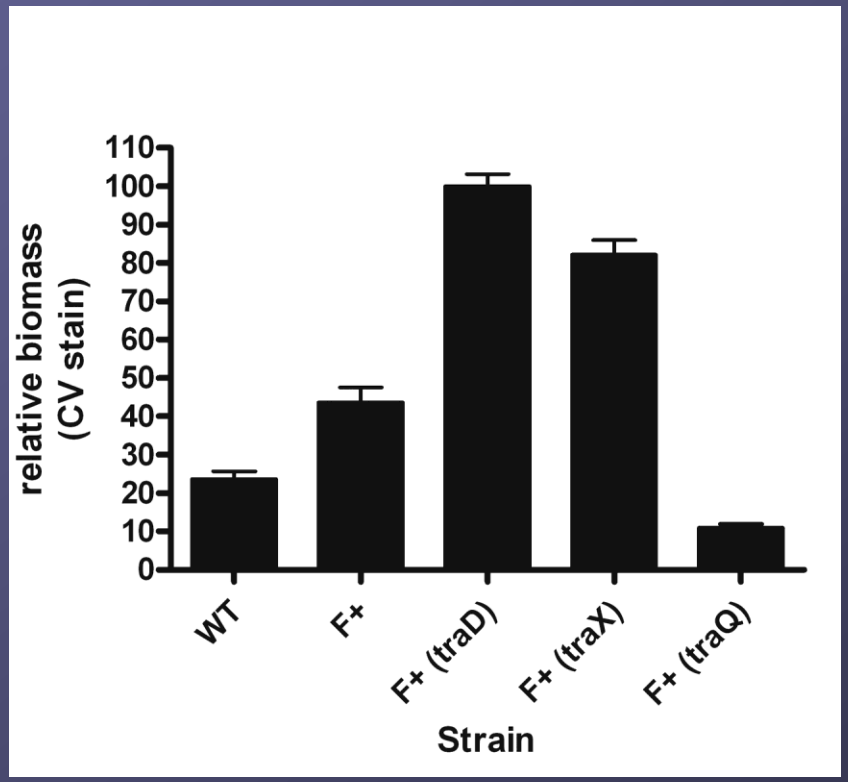
F*traQ*



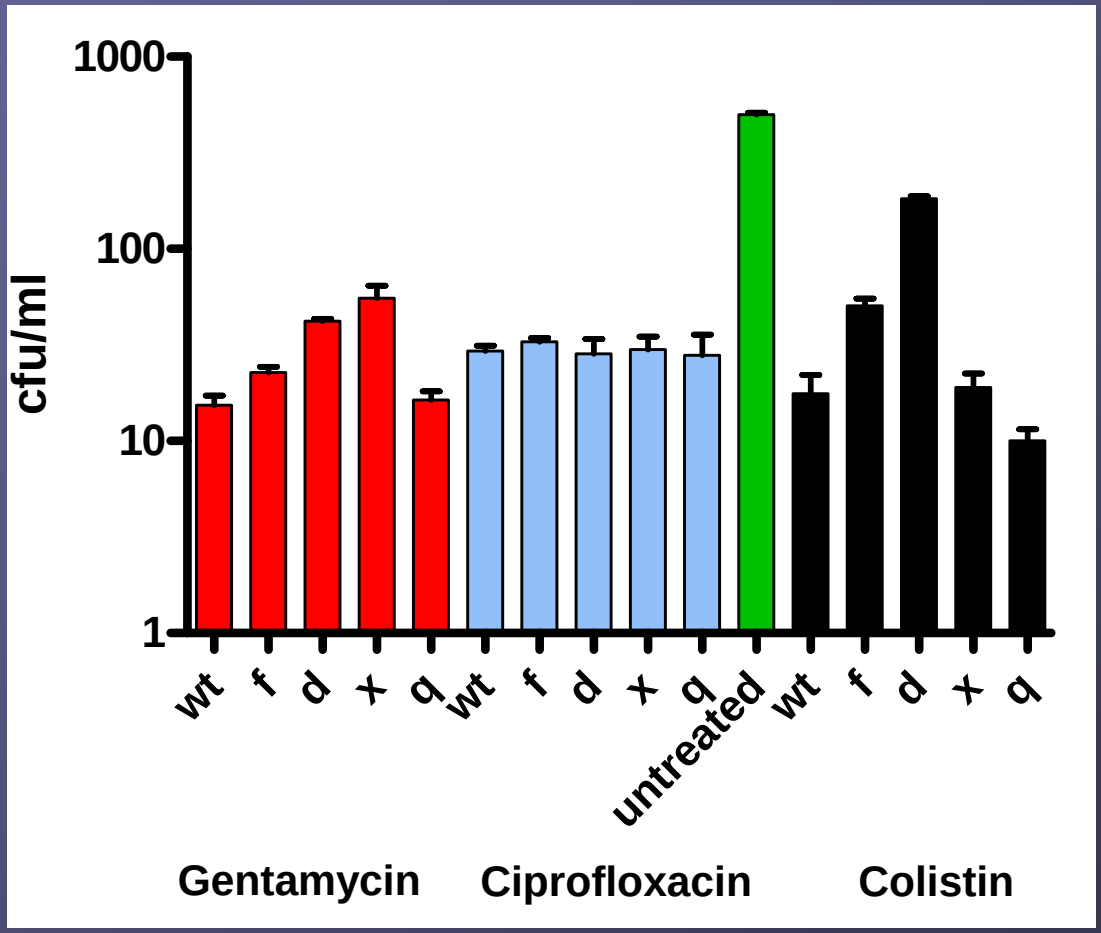
F*traX*



Microtiter biofilm assay CV-staining.



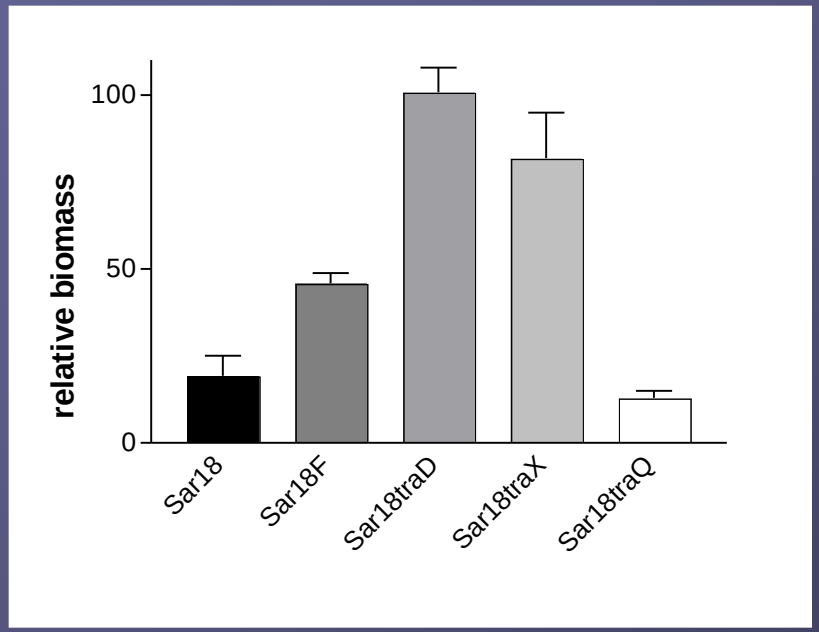
Differential outcomes after antibiotic challenge in *E.coli* biofilms.



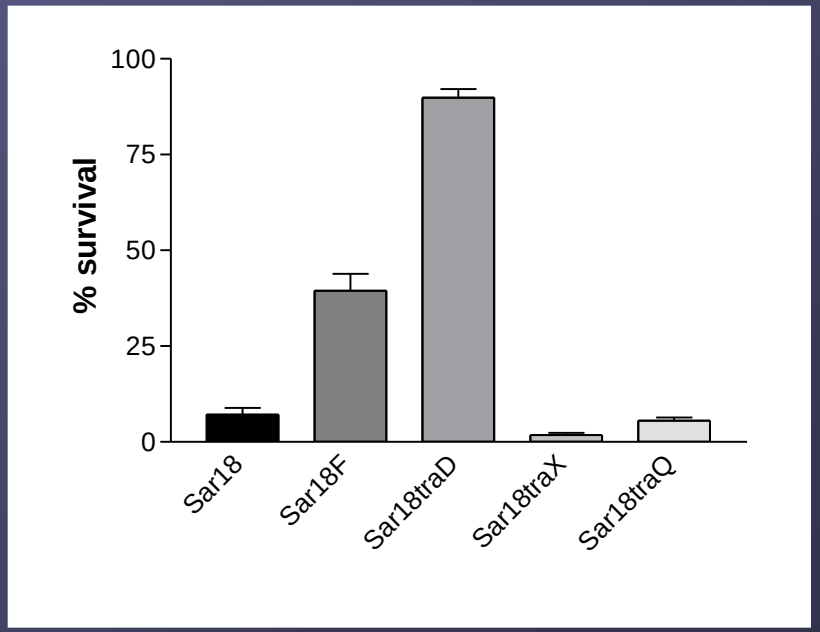
10 x MIC

Differential survival after colistin challenge in *E.coli* biofilms.

Biofilm formation (CV stain)



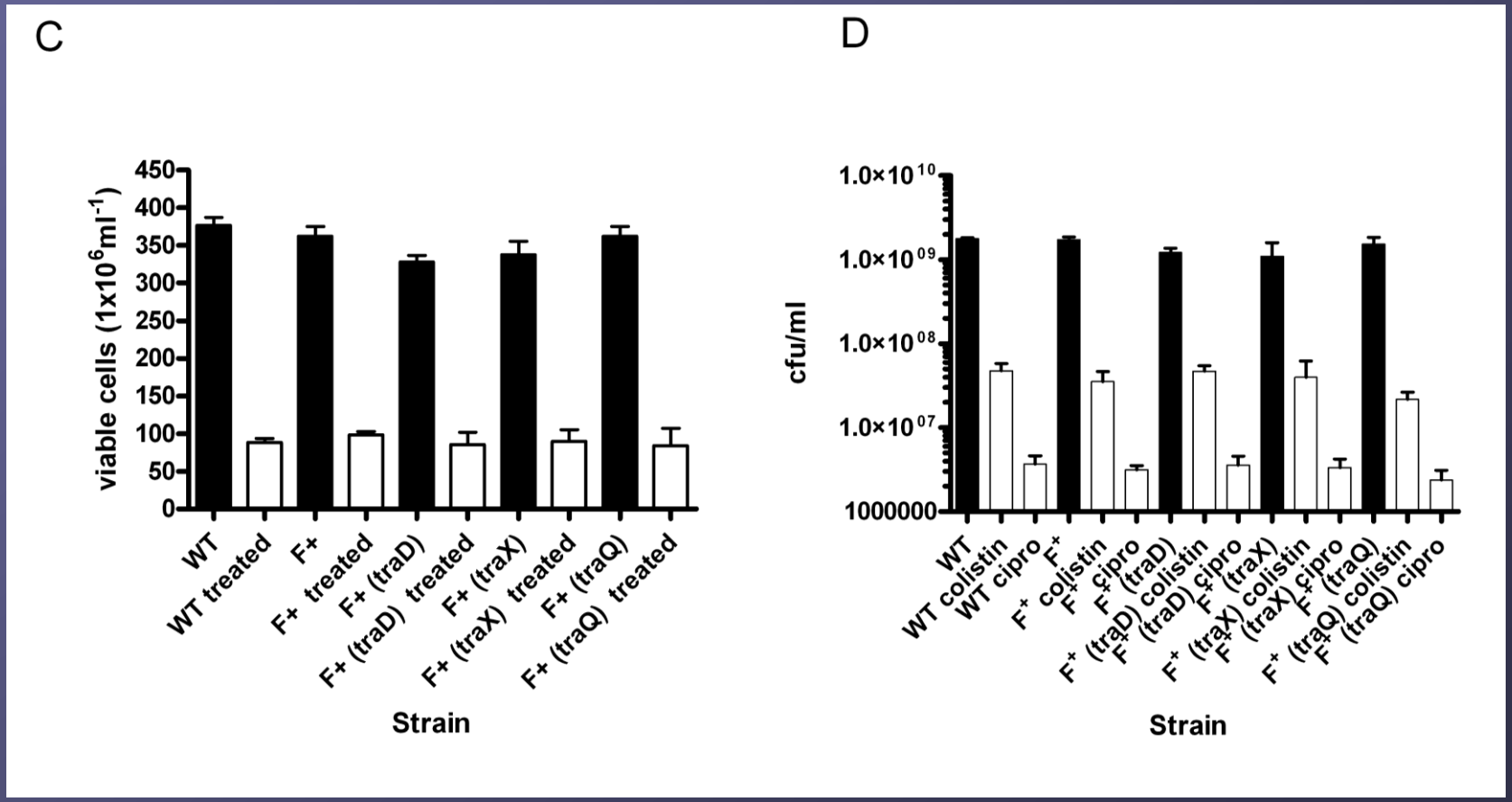
Survival after treatment



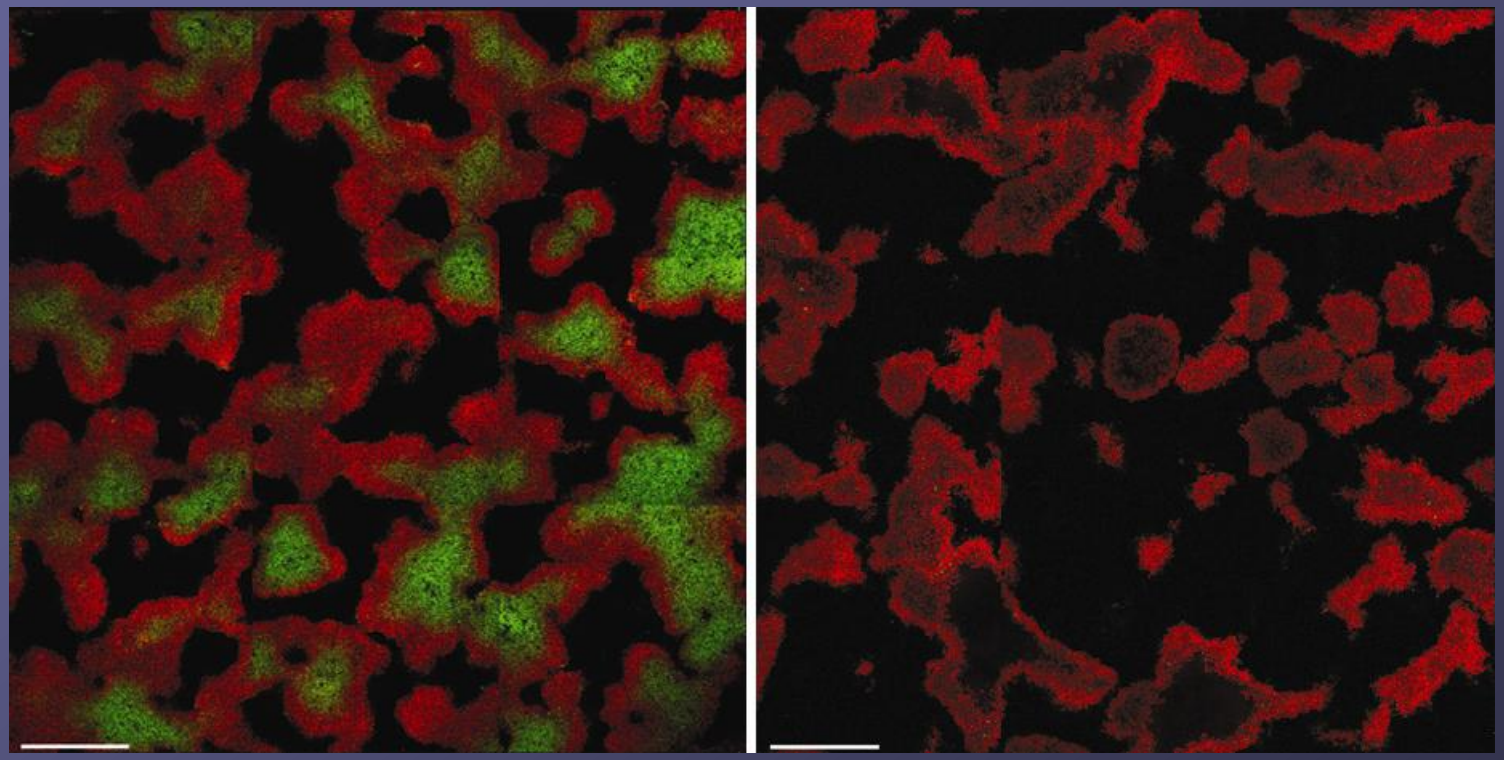
E.coli cells in biofilms differing in organization display differential survival after colistin treatment

Survival Ciprofloxacin

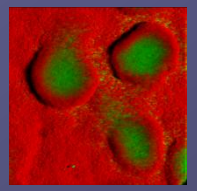
Survival planktonic



A colistin tolerant subpopulation is formed in *E.coli* flow-chamber biofilms.



Biofilm mediated antibiotic tolerance is matter of subpopulation differentiation within the biofilm.

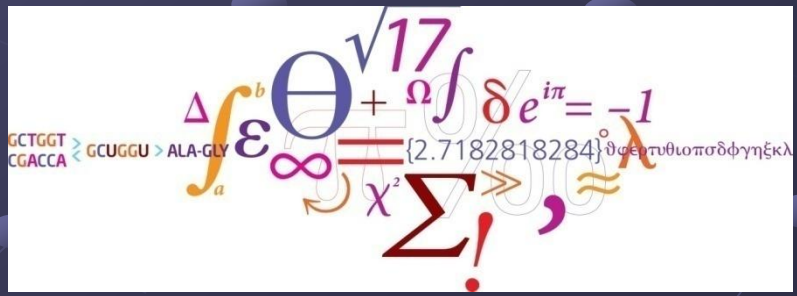


Tolerance of *S. cerevisiae* biofilm to systemic antifungals

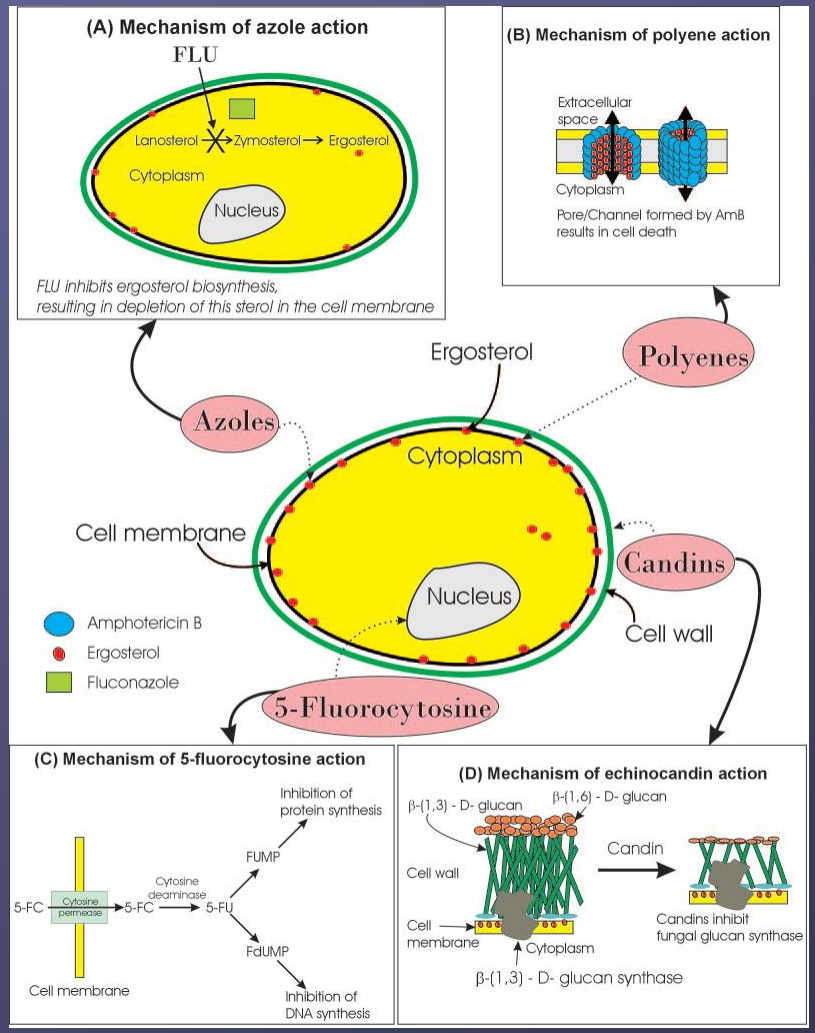
Rasmus Bojsen

Halvårligt biofilmmøde
25.06.12

DTU Systems Biology
Department of Systems Biology

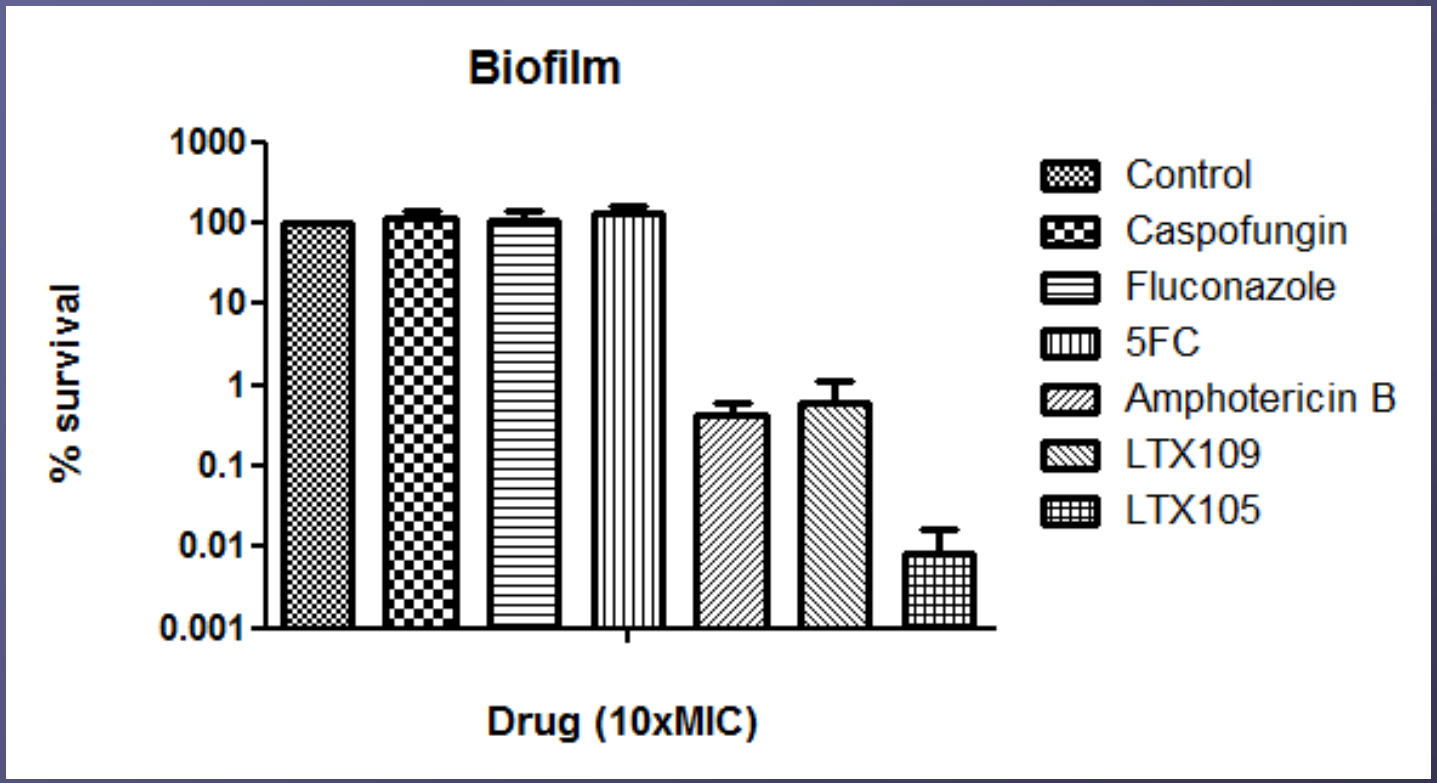


Mechanism of action of systemic antifungals

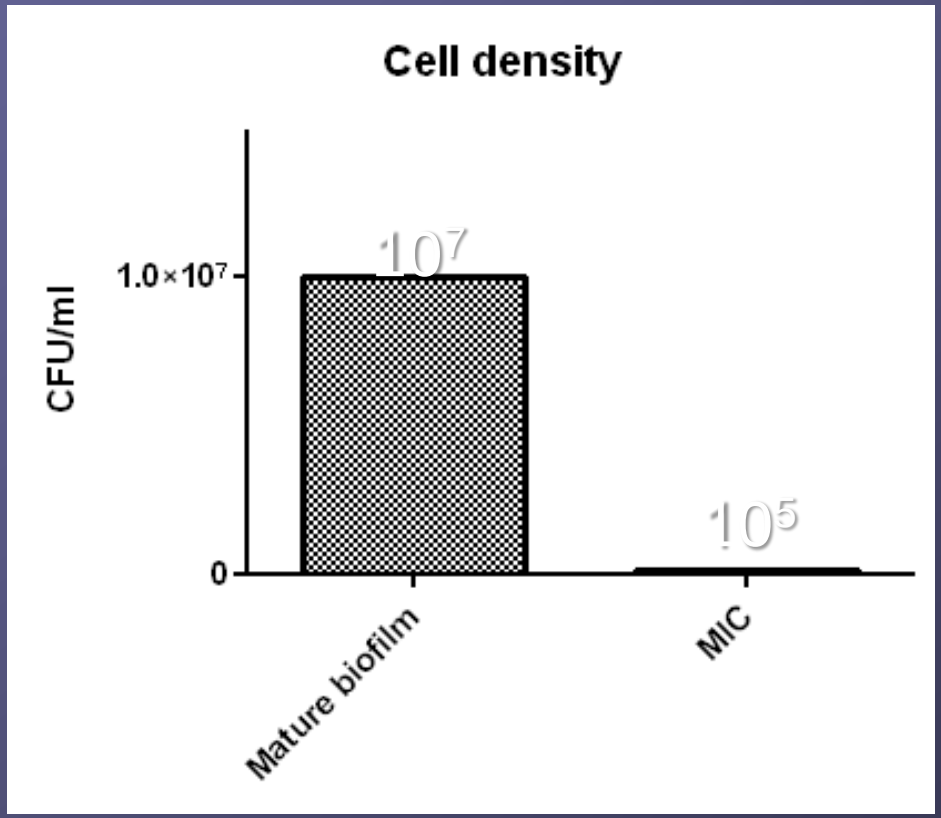


Mukherjee *et al.*, 2005

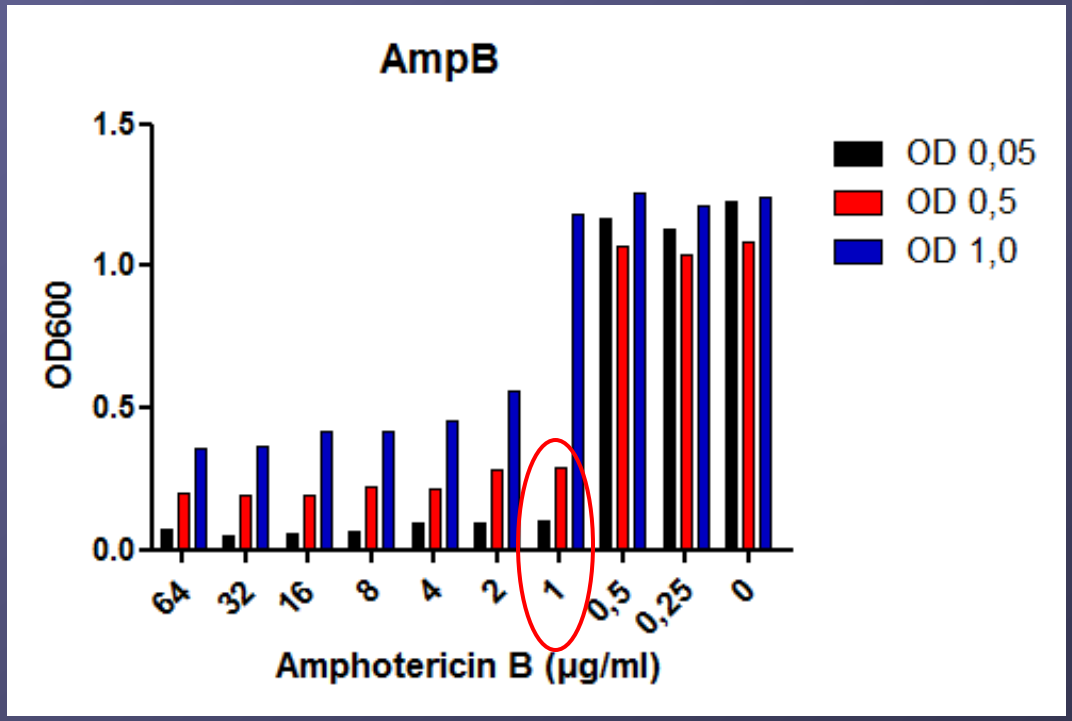
Biofilms are tolerant to antifungals



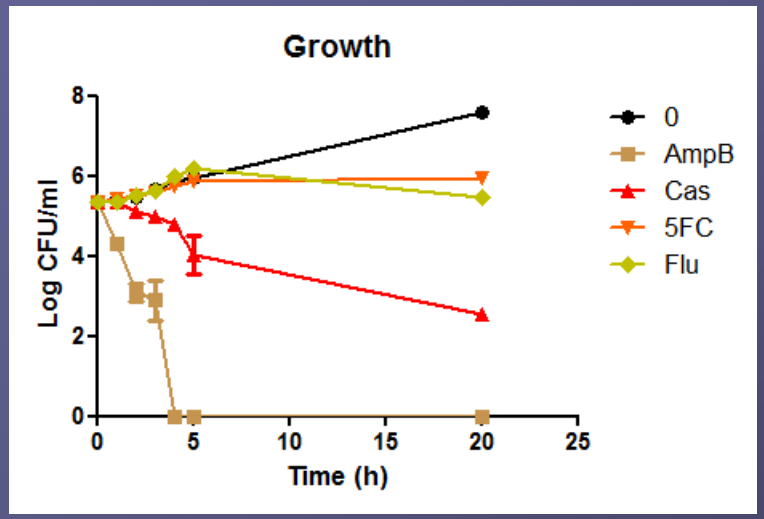
Mature biofilms are in high cell density

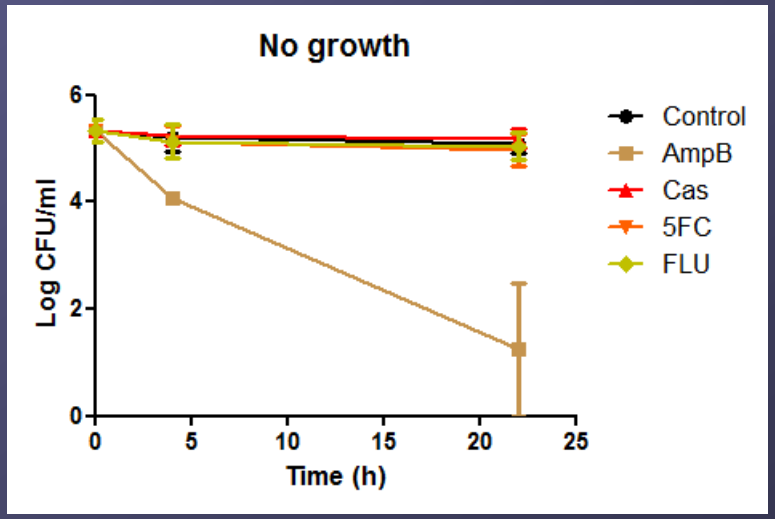


High cell density decrease drug activity

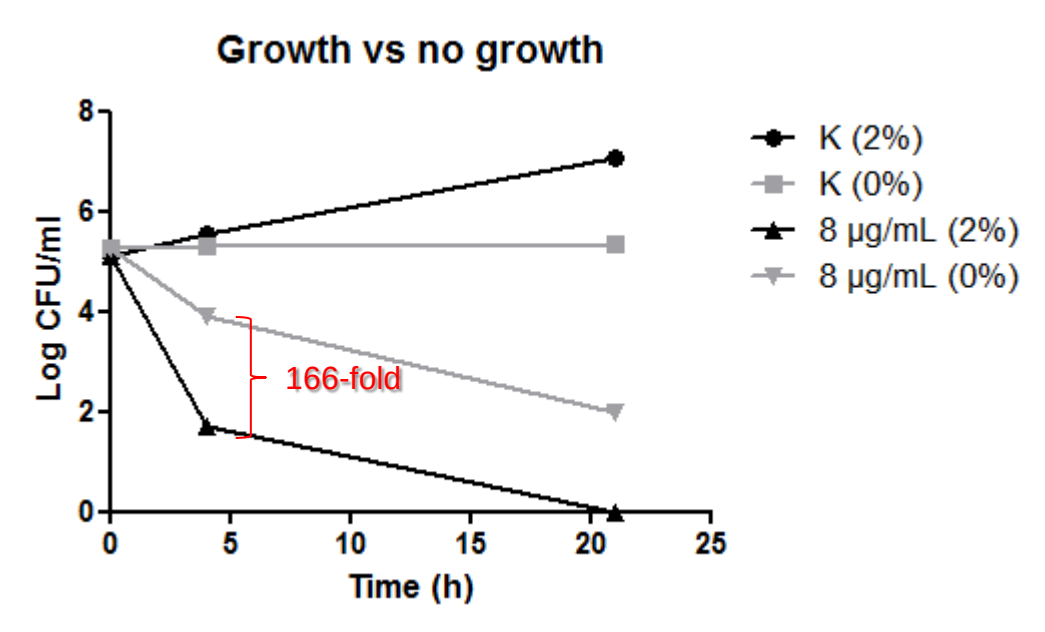


Biosynthesis-acting drugs are ineffective against non-growing cells

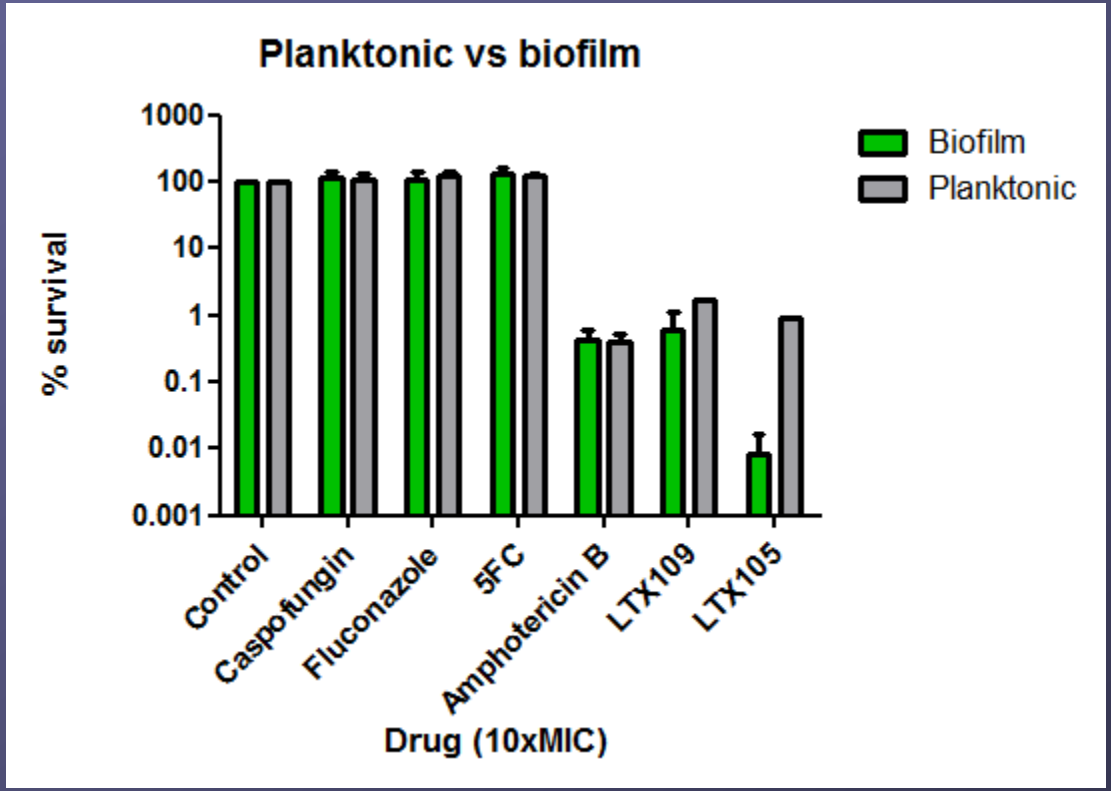




Non-growing cells show increased tolerance to Amphotericin B



Stationary-phase planktonic cells have biofilm tolerance-phenotype

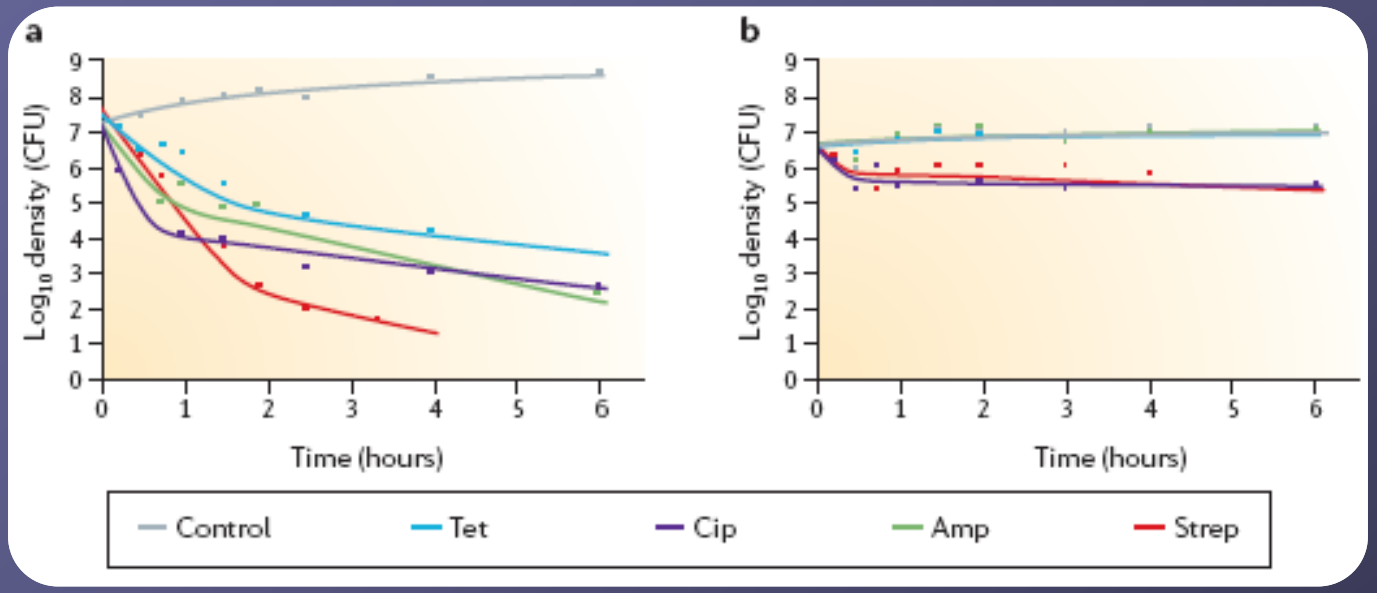


Biofilm mediated antibiotic tolerance is matter of subpopulation differentiation within the biofilm.

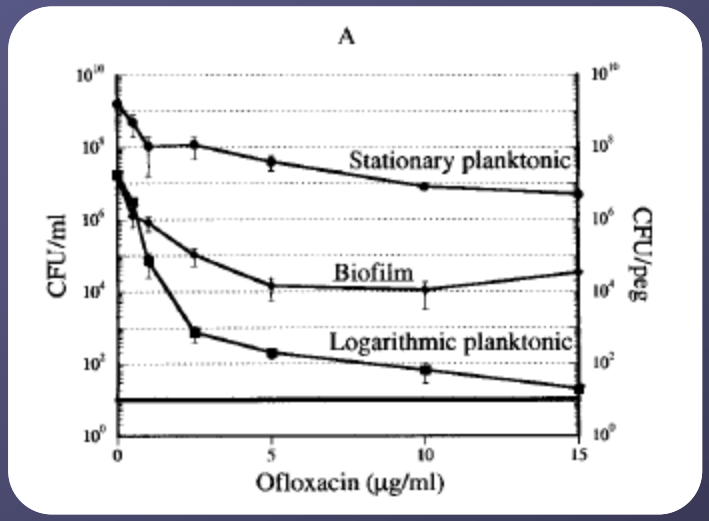
The increased antibiotic tolerance exhibited by biofilm formation is **antibiotic specific** and **conditional**, dependent on the actual **biofilm structure** and on the presence of a **specific** genetically encoded **tolerance mechanism**.

The biofilm mode of growth does not directly predict antibiotic resistance

Biofilm vs stationary phase as an explanation for the failure of AB treatment without resistance.



Levin et.al 2006



Spoering et.al 2001

1. Nguyen, D. et al. Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. *Science* **334**, 982-6 (2011).

Further reading:

1-3

1. Monds, R.D. & O'Toole, G.A. The developmental model of microbial biofilms: ten years of a paradigm up for review. *Trends Microbiol* **17**, 73-87 (2009).

2. Moxon, E.R., Sweetman, W.A., Deadman, M.E., Ferguson, D.J. & Hood, D.W. Haemophilus influenzae biofilms: hypothesis or fact? *Trends Microbiol* **16**, 95-100 (2008).

3. Zuroff, T.R. et al. Robustness analysis of culturing perturbations on Escherichia coli colony biofilm beta-lactam and aminoglycoside antibiotic tolerance. *BMC Microbiol* **10**, 185 (2010).

4. Nguyen, D. et al. Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. *Science* **334**, 982-6 (2011).

How about *Pseudomonas aeruginosa*?

Biofilm systems 4.

Haagensen et.al. J.Bact (2007)

Aim.

The aim of this exercise is to demonstrate how the hydrodynamic
flow chamber biofilm system can be used to monitor gene expression in heterogeneous populations.
We will use a reporter gene fusion
to the *arnB* and *pmrAB* operon inserted in the att Tn7 site to monitor the
response of cells within the biofilm to colistin challenge.

Moreover, we will use a
isogenic regulatory mutant to visualize differential regulation within structured environments.

There are a few question we would like you to pay extra attention to during the exercise:

Where and how is *arnB* expressed?

Where and how is *pmrAB* expressed?

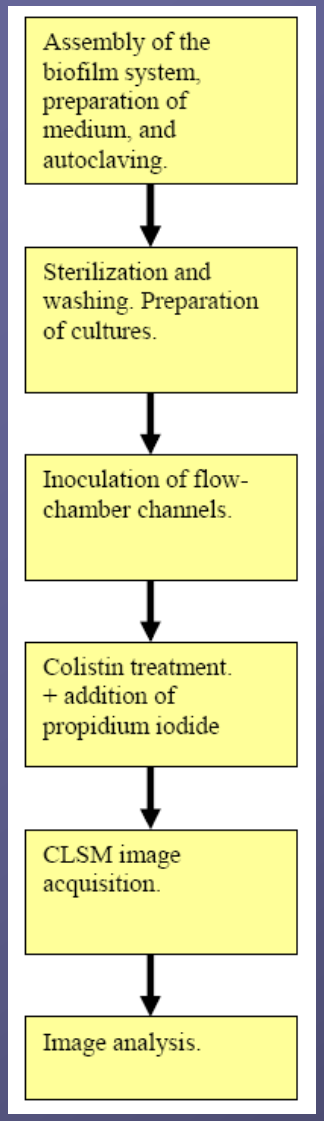
Is there a correlation in *arnB* and *pmrAB* expression?

How does colistin concentration affect the *arnB* and *pmrAB* expression pattern in the biofilms?

What factors are affecting the expression?

What are the strengths and weaknesses of monitoring gene expression flow chamber biofilms?

What are the implications of these results on the study of biofilms and biofilm formation in general?



Minimal inhibitory concentration

E-test

Serial dilution